#11/Election w/C May 6/14/02

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	William Nicol Keitgh	Group Art Unit: 1655
Serial No.:	09/601,267	Examiner: C. Wilder
Filed:	August 25, 2000	
For:	*PROMOTER REGIONS OF THE MOUSE AND HUMAN TELOMERASE RNA COMPONENT GENES*	

May 6, 2002

Commissioner for Patents
Washington, DC 20231

RESPONSE TO THE RESTRICTION REQUIREMENT

Dear Sir:

This is in response to the Restriction Requirement mailed November 5, 2001.

Applicant hereby elects the claims of group I (claims 1-10, 17,18, 26 and 27 for substantive examination in response to the requirement for restriction. This restriction requirement is respectfully traversed. It is respectfully submitted that a search of group I would overlap with a search of the remaining groups, such that it would be more efficient for the USPTO to examine these claims together in a single application than divided among different applications.

In response to the Sequence Listing requirement, substituted sheets 26-30 are submitted concurrently herewith. These sheets add to the figure descriptions SEQ ID NO: x for each sequence shown in the drawings and in the corresponding sequence listing previously submitted.

It is respectfully submitted that this application is in condition for substantive examination, which action is respectfully requested.

Respectfully submitted,

Kenneth D. Sibley
Registration No. 31,665

20792
PATENT TRADEMARK OFFICE

#246006

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on May 6, 2002.

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Brief description of the drawings.

Figure 1 shows the 1765bp genomic nucleotide sequence (SEQ I.D. No. 1) of human telomerase RNA gene (hTR) encompassing the gene promoter region. Transient expression of hTR-reporter gene constructs in HeLa and GM847 cells identified indicate that the elements responsible for promoter activity are contained in a 231bp region upstream of the transcriptional start site.

Figure 2 shows the 4044bp genomic nucleotide sequence (SEQ I.D. No. 2) of mouse telomerase RNA gene (*terc*) encompassing the gene promoter region. Transient expression of *terc*-reporter gene constructs in SWISS3T3 and A9 cells identified the elements responsible for promoter activity are contained in a 73bp region upstream of the transcriptional start site.

Figure 3 shows the restriction enzyme map of the genomic clones encompassing the human and mouse telomerase RNA genes. A) shows human telomerase RNA gene, hTR, genomic structure, 1765bp; B) shows mouse telomerase RNA gene *terc*, genomic structure. The transcribed regions of hTR and *terc* are depicted as black boxes within the central regions of the genomic sequences and the site of the template sequence within the telomerase RNA genes is indicated. The position of the CpG islands are shown as a box beneath the genomic sequence. Numbers in brackets refer to the nucleotide position within the sequence. The 3′-end of all the human promoter fragments is shown as hProm and fragments extend 5-prime to hProm867, hProm697, hProm341 and hProm111. The 3′-end of all the mouse promoter fragments is shown as mProm and fragments extend 5-prime to mProm628, mProm458, mProm418, mProm267,

mProm208, mProm136. The numbers after the prefix, hProm or mProm refer to the number of nucleotides of genomic sequence contained in the promoter fragment.

Figure 4 shows the nucleotide sequence of the human, (a), and mouse, (b), telomerase RNA gene 5'-flanking regions. Putative regulatory motifs are underlined. Arrows indicate the transcriptional start sites, (Blasco *et al.*, 1995; Feng *et al.*, 1995) and numbers to the left of each Figure refer to the number of bases upstream of the transcriptional start site. The template regions are in bold and underlined. Sequences contained in promoter constructs are shown by vertical lines and labelled, hProm or mProm, (see Figures 1 and 3). The regions containing elements responsible for minimum promoter activity are highlighted in bold, (see text for details). The run of CpA dinucleotide repeats in the mouse promoter is shown in bold and italic.

Figure 5 shows the detection of promoter activity in the 5'-flanking regions of human and mouse telomerase RNA genes. For each construct, the length of sequence upstream from the transcriptional start site is shown to the left and the luciferase activity to the right. a) Diagram comparing luciferase activity from human promoter constructs in GM847 and HeLa cells. Data for each construct is plotted as a percentage of the hProm505 luciferase activity as this construct consistently gave the highest activity in human cells. For each construct the mean and standard deviation for duplicate transfected wells is shown. b) Diagram comparing luciferase activity from mouse promoter constructs in SWISS3T3 and A9 cells. Data for each construct is plotted as a percentage of the

mProm458 luciferase activity as this construct consistently gave the highest activity in mouse cells. For each construct the mean and standard deviation for duplicate transfected wells is shown.

Figure 6 shows details of the oligonucleotide primers used for both the human and the mouse sequences. The positions of the primers can been seen on the maps provided in Figures 7 and 8.

Figure 7A shows a map of the 1765bp hTR construct with the position of the primers (see Fig. 6) shown. In each case the percentage homology of the primer is indicated.

Figure 7B shows a map of the 1765bp hTR construct with the restriction sites marked.

Figure 8A shows a map of the 4044bp mouse *terc* construct with the position of the primers (see Fig. 6) shown. In each case the percentage homology of the primer is indicated.

Figure 8B shows a map of the 4044bp mouse terc construct with the restriction sites marked.

Figure 9 shows the sequence of the TR gene promoter from Balb/c clones. The sequence analysis shows that the sequence is identical to that of the P1 sequence (Fig. 4b) apart from minor polymorphisms.

Figure 10 shows potential transcription factor binding sites within the hTR promoter.

Figure 11 shows the basal hTR promoter (-107 to + 69). This is a 176bp region termed sequence 2923 which contains several potential Sp1 transcription regulation sites, retinoblastoma control elements, (RCE) and the promoter CCAAT-box transcriptional regulator site. Sequence elements are defined in Figure 12.

Figure 12 shows oligonucleotides used in the cloning and mutagenesis of the hTR promoter region.

Figure 13 concerns mutagenesis of CCAAT-binding site. It shows a gel shift assay with the h10 sequence for the CCAAT binding site. h10 can bind to the Hela nuclear extract protein and can be competed by itself (h10). Mutation (h10m1) does not compete with oligo(h10) whereas h10m2 still competes. CCAAT site was mutated from CCAA to AGTC leading to loss of binding activity. This suggests that CCAAT site is a functional region for DNA-binding proteins.

Figure 14 shows nuclear factors binding to the Sp1 sites in the hTR promoter. Two separate DNA regions can be recognised by Hela protein extract and produce one Sp1(top) band-shift, two Sp3 (bottom) band-shift. This shows two Sp1 binding sies present in the proximal hTR promoter region.

Figure 15 shows site-directed muatgenesis of the Sp1-2 binding sites. It shows that specific Sp1 binding can be blocked by two bp nuclear changes in the Sp1-2(h41) sequence. 1: no competitor 2: competition by Sp1 consensus sequence 3: competition by Sp1-2 itself 4: no competition by mutation, h41m.

Figure 16 shows site-directed mutagenesis of the Sp1-1 binding region. It shows Sp1-1(h9) is critical region for hTR promoter. A series of mutations were introduced into h9 region by four bp change. Mutation are h9m1, h9m11, h9m2, h9m21 and h9m3. First lane no competitor. Second lane, competition by Sp1-1 itself(h9); fifth and six lanes show no competition by mutation m2 and m21; third, forth and seventh lanes show competition by mutation 1, 11 and mutation 3. This suggests that mutation m2 and m21 base

pair region responsible for DNA-binding protein activity. Mutation of TATA-box still competes wild type DNA-binding.

Figure 17 concerns functional analysis of promoter mutants. It shows the scheme for introducing specific mutations into the hTR promoter. In brief, the mutant fragments were cloned into the luciferase basic vector, and the mutant confirmed by sequence. Mutant constructs were then transfected into 5637 cells to analysis luciferase activity. 5637 cells are a bladder carcinoma cell line which is p53 and pRb negative.

Figure 18 shows deletion analysis of the 5' flanking region of the hTR gene in Hela cells. The wild type promoter sequence used in this study is the hTR 2923 sequence shown in Figure 11. The promoter activity of this clone relative to the previously described promoter clones, (see Fig 5a), is shown in Figure 18.

Figure 19 concerns scanning mutation analysis of the hTR proximal promoter region (-107 to +69). It shows constructs with hTR promoter sequence element mutations. The parental wild type sequence is called 2923 and mutant constructs are shown below.

Figure 20 shows a diagrammatic version of the mutant promoter constructs described in Figure 19, which were used to assay for promoter activity.

Figure 21 concerns mutation analysis of the hTR promoter in 5637 cells. It shows the ability of the mutant promoters to drive gene expression in comparison to the wild type promoter sequence in cell lines. Left side shows the mutation constructs, right side shows luciferase activity relative to wild type(2923). Mutation Sp1-2 decrease the basal activity. Mutation Sp1-1 or both Sp-1